Atypical mitochondria in the ellipsoid of the photoreceptor cells of vertebrate retinas

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The fine structure of ellipsoids in a number of vertebrate retinas was studied in material prepared with various fixatives. Several atypical ellipsoidal mitochondria were found in ellipsoids of certain animals. A peculiar configuration of the internal mitochondrial membranes caused by fusion of the outer surfaces of adjacent cristae was found in the lamprey retina. Intramitochondrial glycogen granules and granular or filamentous intracristal inclusions were observed in the toad and giant salamander, respectively. In photoreceptor cells of the gecko, carp, and snake, the ellipsoid was particularly differentiated, containing modified mitochondria. The possible functional significance of such differentiated ellipsoids is discussed briefly with respect to the oil droplets.

Key words: retinal ellipsoid, mitochondria, ultrastructure, electron microscopy, glutaraldehyde, osmium tetroxide, glycogen.

The ellipsoid of photoreceptor cells is located at the apical region of the inner segment and appears to be homogeneous in fresh material. It was first described as a dense aggregation of elongated mitochondria by Sjöstrand in his pioneering electron microscope study, and this has been found to be the typical conformation of the ellipsoid in vertebrate retinas. During the course of a comparative study on the retinal fine structure of a wide variety of vertebrates, it was noted that ellipsoidal mitochondria exhibit great variation in structural details. Although a number of atypical forms of mitochondria, or those possessing various inclusion bodies, have been reported in many cell types, atypical and specially modified ellipsoidal mitochondria found in certain animals seem to be quite unique. In this paper the most unusual types of ellipsoidal mitochondria will be described, and their possible functional significance will be discussed briefly.

Materials and methods

Although a number of animal species from cyclostomes to mammals, mostly adult, have been used in the course of a comparative study on the retinal fine structure in our laboratory, the observations described in this paper were restricted to those animals which contain the most unusual ellipsoidal mitochondria; they are: lampreys, carp, giant salamanders, snakes, geckos, turtles, and pigeons.

Since the electron microscopic image of mitochondria varies, depending upon the methods used to fix and stain them, at least two kinds of fixatives with different ingredients were utilized for the fixation of each animal retina. In general, the retina, with or without the choroid and sclera, from one eye of an individual animal was fixed in one per cent osmium tetroxide, and the retina from the other eye was fixed in 3 to 6 per cent glutaraldehyde followed by a 2 hour fixation of osmium. The fixatives were buffered with Millonig's...
phosphate, s-collidine, or sodium cacodylate. All of the fixed retinas were dehydrated in either a graded series of ethanol or acetone, and embedded in Epon. Ultrathin sections were cut mainly with glass knives or a diamond knife on a Porter-Blum microtome, stained with lead tartrate or lead citrate, or 2 per cent aqueous solution of uranyl acetate, or a combination of both. Some of the material was stained for one hour in a block with aqueous uranyl acetate before dehydration.

One micron sections of Epon-embedded tissue were obtained, cut with the same microtome, and stained with toluidine blue for histological examination. Periodic acid–Schiff reaction or Sudan black B staining was also utilized in some cases.

Observations

Generally speaking, ellipsoids of vertebrate photoreceptor cells consist of a dense aggregation of mitochondria. The shape and size of these, however, vary considerably from one species to another, as do the other types of cells and tissues. In addition, unusual forms of mitochondria and specially modified ones are also found. For convenience, they will be described here divided into several groups according to the patterns of their structural modification.

A very unusual form of internal mitochondrial membrane was encountered in the ellipsoids of the lamprey, *Entosphenus japonicus*. In this retina, two types of visual cells, rods and cones, have been described. In an ordinary histologic preparation, the ellipsoidal region of receptor cells appears in two parallel rows.

Fig. 1 is an electron micrograph of the lamprey retina, showing the ellipsoids of the so-called rod photoreceptor cells. Mitochondria, having an elongated shape with rather sparse cristae located vitreally, are typical of ellipsoids. However, the mitochondria gradually increase in size toward the scleral end of the ellipsoid. Such mitochondrial gradient in size is not infrequent in the ellipsoid of lower vertebrates, particularly in cone ellipsoids, while those of human photoreceptor cells, or of rod cells in most animals, consist of a homogeneous population of mitochondria. With the increase in mitochondrial size, cristae also increase in number and are organized in parallel array along the long axis of mitochondria. In larger mitochondria with an irregular contour, they are sometimes arranged in groups which are distributed at random within a mitochondrion. In some cases, cristae in lamellar patterns having sharp angulations of the membranes present a zigzag course in the same way described in some other tissues (Fig. 2).

A peculiar configuration of internal mitochondrial membranes can occur in these parallel arrayed cristae and is shown by arrows in Figs. 1 and 2. Closer examination of this peculiar structure reveals that certain numbers of parallelly arrayed cristae adhere tightly to each other and two membranes of adjacent cristae appear to fuse into a dense membrane of approximately 110 Å in thickness. Consequently, intercristal space or mitochondrial matrix disappears in this region. On the other hand, the thickness of intracristal spaces of such cristae diminishes by approximately half, but the spaces never vanish completely. Such membrane fusions were encountered only between the internal mitochondrial membranes, and were never observed between different mitochondria or between an outer and inner mitochondrial membrane. Fig. 3 shows the peculiar configuration of internal mitochondrial membranes sectioned in a plane nearly normal to the structure and demonstrates its finer detail.

Other variations are atypical ellipsoidal mitochondria which contain inclusion bodies or material in the lumen of cristae.

Ordinarily intracristal space is fairly constant and is about 60 to 50 Å in width. In this group of ellipsoidal mitochondria, however, this space is dilated locally and contains either granular or amorphous materials. As previously reported, in the photoreceptor cells of the rat retina, the granular inclusion in the lumen of cristae was identified as glycogen. Similar intramitochondrial glycogen granules were also found in subsequent studies in retinal ellipsoids of toads, *Buto vulgaris japonicus* (Fig. 7), the snake, and the carp. It is noteworthy that glycogen-containing mitochondria in retinal tissue were always found in rods.
An example of amorphous intracristal inclusion of mitochondria was observed in the ellipsoid of the giant salamander retina, in which rod cells are predominant. Fig. 4 shows a vertically sectioned rod cell of a giant salamander, *Megalobatrachus japonicus*. The ellipsoid consists of closely packed, rather uniform-sized mitochondria. A number of these intracristal spaces are dilated in places and filled with a dense amorphous material. It seems likely that further accumulation of this material enlarges not only the lumen of the crista, but also the mitochondrion itself.

Although most intracristal material appears in a very fine granular form under high power (Fig. 5), filamentous inclusions with a helical substructure were found in some instances (Fig. 6). Very similar structures have been reported in astrocytic mitochondria of the rat.6

The third group of ellipsoidal mitochondria is very unique and is regarded as an especially differentiated form of the ellipsoid. Morphologically, we can distinguish two types in this group, one of which was found in the retina of snakes and the other in carps and geckos.

In this study the retinas of two species of snakes, *Elaphe climacophora* and *Agkistrodon hylas blomhoffii*, were examined. The retina of the former belongs to the so-called pure cone type, and the latter contains both rods and cones. In both species there are single and double cones. Single cones and the principal member of the double cone are morphologically identical and both possess a specially differentiated ellipsoid, which has a barrel-shaped body with a densely staining core, as shown in Fig. 8. The ellipsoid of the accessory member of the pair consists of ordinary mitochondria. In this paper the description will be restricted to the differentiated type of ellipsoid.

Fig. 9 is a transverse section of a single cone in *Elaphe* retina at the level of the ellipsoid. Numerous mitochondria are...
Figs. 1–3. For legend see opposite page.
Fig. 4. For legend see p. 304.
Figs. 5–7. For legend see p. 304.
packed closely and in a fairly orderly manner, that is, small typical ellipsoidal mitochondria about 0.3 μ in diameter at the periphery, and the larger unusual ones more centrally. The mitochondria sometimes contain small osmiophilic granules. These granules increase in number and size (up to 0.2 μ in diameter) in the mitochondria located more centrally, and are arranged regularly along the mitochondrial membranes or cristae (Fig. 10). Therefore, one can discern more easily the irregular outlines of mitochondria by tracing the granules which are closely arranged in a row.

Depending on fixation or treatment with saliva and RNase, the electron density or shape of these intramitochondrial granules changed very slightly, nor was their size, number, or pattern of distribution influenced. The snake ellipsoids, with such unusual mitochondria, stained intensely with toluidine blue and showed a positive reaction for Sudan black B staining in histological preparations.

Another type of unusual ellipsoid was found in one of the paired receptor cells in the gecko retina and in cone cells of the carp retina.

Although further histological study is

**Fig. 8.** A nearly vertical section of a snake pure cone retina. Single cone (SE) and the principal member of double cone (PE) have a large, barrel-shaped ellipsoid with a dense, granulated core. On the other hand, the ellipsoid of an accessory cone (AE) consists of ordinary mitochondria. Pe, Pigment epithelium; EM, external limiting membrane; OS, outer segment. Fixed in OsO4-Millonig’s buffer, stained with lead tartrate. (Original magnification ×4,000.)

**Fig. 9.** Cross-sectioned ellipsoid of a snake single cone. Typical ellipsoidal mitochondria are located at the periphery of the ellipsoid. The more central mitochondria are larger and contain small, osmiophilic granules. These granules are larger and more numerous in the mitochondria toward the center of the ellipsoid. Fixed in OsO4-Millonig’s buffer, stained with lead tartrate. (Original magnification ×13,000.)

**Fig. 10.** Part of a modified mitochondrion of the snake ellipsoid at higher magnification. The matrix of the extra large mitochondria is occupied by dense granules which are arranged in rows along the mitochondrial membranes. mm, Outer and inner mitochondrial membranes; cm, cristal membranes. Fixed in OsO4-Millonig’s buffer, stained with lead tartrate. (Original magnification ×64,000.)

**Fig. 11.** Modified mitochondria seen in cone ellipsoids of the carp retina. Large, irregular-shaped mitochondria are almost completely converted to dense solid structures which are surrounded by mitochondrial double membranes (mm). In such mitochondria, only rudimentary or small numbers of cristae (cm) can be seen along the mitochondrial membranes. Fixed in glutaraldehyde-OsO4-Millonig’s buffer, stained with lead tartrate. (Original magnification ×37,000.)

**Fig. 12.** "The Class B double" type photoreceptor cells of the gecko cut transversely through a differentiated ellipsoid. Comparing an ordinary ellipsoidal mitochondrion with that of an accessory member (AE). The ellipsoid of the principal member of the double cone contains a large, lipoidlike structure in the center region. Fixed in OsO4-s-collidine buffer, treated with uranyl acetate in toto before dehydration, and stained with lead tartrate. (Original magnification ×6,000.)

**Fig. 13.** Part of a differentiated ellipsoid of the gecko illustrating gradual alteration from the typical mitochondrion to the lipoidlike structure. This electron micrograph shows that the lipoidlike structure at the center of ellipsoid is an extremely modified mitochondrion. Fixed in OsO4-s-collidine buffer, treated with uranyl acetate in toto before dehydration, and stained with lead tartrate. (Original magnification ×25,000.)

**Fig. 14.** Low power electron micrograph of a turtle retina. In glutaraldehyde-fixed material, oil droplets (OL) appear to be round or oval with very smooth outlines. Pe, Pigment epithelium; OS, outer segment; E, ellipsoid; P, paraboloid. Fixed in glutaraldehyde-OsO4-Millonig’s buffer, stained with lead citrate. (Original magnification ×5,000.)

**Fig. 15.** Material from the same retina, as shown in Fig. 14, but fixed in osmium tetroxide. In sharp contrast to Fig. 14, the oil droplet has a very irregular contour. Its surface is applied closely to the surrounding mitochondria which have densely packed cristae. Fixed in OsO4-Millonig’s buffer, stained with lead citrate. (Original magnification ×19,000.)
Figs. 8 and 9. For legend see opposite page.
Figs. 10 and 11. For legend see p. 308.
Figs. 12 and 13. For legend see p. 308.
Figs. 14 and 15. For legend see p. 308.
needed, the photoreceptor cells of *Gekko japonicus* can be divided approximately into two types. One appears as a twin or triplet in which all members are equal and ordinarily have a typical cone ellipsoid. Another type is a pair of unequal cells with exactly the same structure as ordinary double cones except for the size and shape of the outer segment. According to Underwood,7 these 2 types of visual cells may correspond to the Class C double and the Class B double cones, respectively. A specifically differentiated ellipsoid dealt with in this paper was found in one of the Class B double types.

Fig. 12 is a cross section of gecko retina at the level of the differentiated ellipsoid of the double receptor. Similar to the ellipsoidal pattern of the snake mentioned, the structural feature of these ellipsoids differs between the central and peripheral zones. The first is occupied by large homogeneous bodies with considerable electron density. They are more similar to a lipid droplet. In both vertical and transverse sections, they appear roughly spherical and extend from the central to the distal region of the ellipsoid. However, closer examination reveals that such spherical bodies are surrounded by double membranes with rudimental cristae. In addition, transitional or intermediate forms are observed between typical mitochondria at the vitreal end and spherical bodies at the scleral side (Fig. 13). In other words, with the gradual loss of the cristae, the density of the mitochondrial matrix increases, suggesting the accumulation of some unusual material. These facts indicate that the dense spherical bodies are an extremely modified form of ellipsoidal mitochondrion in which the majority of the original mitochondrial components have been replaced by a special substance. Exactly similar structures were found in the cone receptor ellipsoids of the carp, *Cyprinus carpio* (Fig. 11).

Finally, observations on the photoreceptor cells with oil droplets will be described briefly. Several variations of oil droplets, colored or colorless, are located at the scleral end of the cone inner segment of a great variety of vertebrates.8

Figs. 14, 15, and 16 are electron micrographs of receptor cells with oil droplets in turtle and pigeon retinas. One can easily distinguish these droplets by their various densities. Some are quite dense while others are less so, which probably represents differences in their composition that are responsible for their color which is recognizable in the fresh material.

The most interesting feature of the oil droplets is their shape. In osmium-fixed material, the oil droplets have a rather irregular contour, whereas those prefixed with glutaraldehyde are round or oval with a very smooth outline. Such sharp contrast in shape was well demonstrated in the oil droplets of the turtle, *Clemmys japonica*, shown in Figs. 14 and 15.

Regardless of the manner of preparation, the ellipsoid substance is in intimate contact with an oil droplet, mainly on its vitreal surface. As seen in Fig. 15, the mitochondria sometimes appear to adhere closely to oil droplets so that the outer mitochondrial membrane in the region of contact seems to have disappeared.

Ellipsoidal mitochondria accompanied by oil droplets were typical in all cases. They had densely packed cristae and no structural modification or other observable differentiation.

**Discussion**

The mitochondria in retinal ellipsoids, for example, those in human photoreceptor cells, are typically long and slender and are arranged in rows parallel to the axis of the inner segment. However, there are several variations in vertebrate retinas. In some cases, the variation appears in one individual mitochondrion in an ellipsoid, and in others, the ellipsoid as a whole is differentiated into regions characterized by special mitochondria. The ellipsoidal mitochondria in the lamprey, giant salamander, and toad belong in the former category, while the gecko, carp, and snake ellipsoids belong in the second.
Fusion occurring between membranes of the cristae themselves and the inner mitochondrial membrane in the lamprey ellipsoid resembles somewhat the fine structure of grana in the chloroplast of plant cells. Similar structures have not been reported so far in any mitochondria. They are found in nearly all receptor cells in each lamprey retina so far examined, regardless of the methods of fixation employed. Therefore, this structure may hardly be regarded as an artifact.

As is shown in Fig. 3, the fusion occurred always between the outer surface of adjacent cristae, or between the mitochondrial inner membrane and the outer surface of cristae; therefore, the intercristal spaces disappeared in these places. No membrane fusions were recognized involving the outer mitochondrial membranes. These findings may indicate an asymmetrical structure of the outer and inner mitochondrial membranes. The question is raised whether there is any change in the macromolecular composition of such fused regions. Are there any elementary particles in the region of such fused membranes? These are unsolved problems.

Whereas the granulated inclusion bodies in the intracristal lumen of ellipsoidal mitochondria of certain photoreceptor cells were identified as glycogen, the chemical
nature of the intracristal amorphous substance and filaments with helical substructure seen in giant salamander ellipsoids is unknown. The intramitochondrial glycogen granules, as well as intramitochondrial inclusions in various forms, filamentous or crystalline, have been reported in a number of different cell types and organisms.9–11 The presence of DNA fibrils12 or insoluble inorganic salts13 has been well established recently. Thus, accumulation of special substances in mitochondria would not be specific features for ellipsoidal mitochondria, but rather common phenomena for mitochondria in general. However, the functional significance of the intracristal material in the ellipsoidal mitochondria is still unknown.

In contrast, the characteristic ellipsoids of receptor cells in the gecko, carp, and snake can be assumed to be a specifically differentiated form with relation to visual perception. The fine structures of the retinal oil droplets, reported by several authors, can be classified into two types. In Type I, the oil droplet is more like the lipid droplets in other cell types; it shows a homogeneous internal structure with various densities, such as those in the frog,14 diurnal gecko,15 and pigeon.16 Type II is one which was reported by Berger17 in the guppy double cone inner segment. It is bounded by a double membrane, and is vesicular or granular in internal structure. In his paper Berger concluded that mitochondria at the scleral end of ellipsoids differentiate into oil droplets. However, the observation on the developing chick retina which contains Type I oil droplets in mature form reveals: (1) that oil droplets are already found as small droplets at early developmental stages when the apical region of visual cells has started to differentiate into inner segments, and (2) that direct transformation from mitochondria to oil droplets, as described in the case of the guppy, is not apparent throughout the developmental stages of photoreceptor cells in the chick, despite the close topographical relationship between them (unpublished observation).

Thus, oil droplets of Type I and Type II are entirely different entities in their structure, as well as in their morphogenesis.

The structural features of the modified mitochondria in the specially differentiated ellipsoids of the gecko, carp, and snake are similar to those associated with the Type II oil droplet described by Berger. Since such modified mitochondria are still bounded by a double membrane, possess rudimental cristae, and are intermediate between the typical mitochondria and the modified ones, whether it is reasonable to designate them as "oil droplets," as was done in Berger's paper, is still questionable. However, numerous intramitochondrial granules in the snake photoreceptor ellipsoid contain a Sudan black B positive substance, and the modified mitochondria in the gecko and carp have structural similarity to lipid droplets in many cell types. In addition, no ordinary oil droplets exist within such a differentiated ellipsoid. These findings lead us to speculate that such ellipsoids as a whole may function as oil droplets, since each modified mitochondrion maintains its individuality in the ellipsoid. More specifically, the ellipsoid of certain animal visual cells may be assumed to have a dual functional significance, namely, as a source of energy for the photoreceptor cell, and to function as an oil droplet.

REFERENCES
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